Review of the Evidence Relating to Glyphosate and Carcinogenicity

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Introduction

Glyphosate (N-phosphonomethyl glycine; CAS registry #1071-83-6) is the primary active ingredient in many generic herbicides. Glyphosate is formulated primarily as an isopropylamine, ammonium, or sodium salt in water soluble concentrates and water soluble granules. The relevant impurities in glyphosate technical concentrates are formaldehyde, N-nitrosoglyphosate and N-nitroso-N-phosphonomethylglycine. Surfactants and sulfuric and phosphoric acids may be added to formulations of glyphosate, with type and concentration differing by formulation. The United States (US) Environmental Protection Agency (EPA) and other regulatory agencies around the world have registered this chemical as a broad-spectrum herbicide for use on multiple food and non-food use crops. Glyphosate-based herbicides, which have been sold in the US since 1974, are now registered in over 130 countries.

Glyphosate is widely considered by regulatory authorities and scientific bodies to have no carcinogenic potential. The US EPA (1993) has classified glyphosate as a Group E carcinogen, which is defined as having "evidence of non-carcinogenicity for humans". This classification was based on "a lack of convincing evidence of carcinogenicity in adequate studies with two animal species, rat and mouse". Negative results were observed in genotoxicity studies that were conducted under good laboratory practice conditions and compliant with contemporary regulatory test guidelines.

However since that time, results of further studies have come to light, and the International Agency for Research on Cancer (IARC) Monograph 112 on glyphosate (released on 29 July 2015) came to the conclusion that glyphosate should now be classified as a carcinogenic substance in Group 2A (probably carcinogenic to humans). This classification was based on "limited evidence" from human data (regarding non-Hodgkin lymphoma (NHL)) but "sufficient evidence" in animal-experiments. The rationale identifies that the IARC working group (IWG) also notes mechanistic and other relevant data in support of the conclusion; in particular the IWG cites "strong evidence" that glyphosate can operate by two key characteristics of known human carcinogens, namely genotoxicity and oxidative stress.

This classification was initially published in a short report by Blair et al, (2015) in the "Lancet Oncology" on 20 March 2015.

This report discusses the relevant data on glyphosate, especially the more recent studies, and reviews the basis on which the IWG classified it as a probable human carcinogen (Group 2A). This involves review of the quality of evidence for carcinogenicity in humans and experimental animals and the mechanistic arguments.

Cancer in humans

The IWG found there was limited evidence in humans for the carcinogenicity of glyphosate. Some case-control studies of occupational exposure in the USA, Canada, and Sweden reported increased risks for NHL that persisted after adjustment for other pesticide exposures. However the Agricultural Health Study (AHS) cohort did not show a significantly increased risk of NHL. These studies are discussed below.

Case-control studies in the Midwest USA

Three case-control studies were conducted by the U.S National Cancer Institute in Iowa and Minnesota in the1980s using the same control series, but each investigating a different lymphohaematopoietic cancer. Brown et al, (1990) found a near null association between

glyphosate exposure and leukaemia among white males residing in the area (OR = 0.9; 95% CI 0.5–1.6). Among Iowa farmers reporting ever handling glyphosate, there was a slight non-statistically significant odds ratio for multiple myeloma (OR = 1.7; 95% CI 0.8–3.6) (Brown et al, 1993). Cantor et al, (1992) found an approximately null association between glyphosate exposure and NHL among males (OR 1.1; 95% CI 0.7–1.9).

The IWG reviewed a later study by De Roos et al, (2003) who used pooled data from three case-control studies of NHL conducted in the 1980s in Nebraska (Zahm et al, 1990), Iowa and Minnesota (Cantor et al, 1992), and Kansas (Hoar et al, 1986). Reported use of glyphosate as well as several other individual pesticides was associated with an increased risk of NHL. A total of 650 cases and 1,933 controls were included for the analysis of 47 pesticides. Reporting glyphosate exposure were 36 cases and 61 controls. After adjusting for other pesticide use, age, and study area, by two regression techniques, odds ratios of 2.1 (1.1–4.0) using logistic regression and 1.6 (0.9–2.8) using hierarchical regression were found.

In that regard, a later study by De Roos et al, (2005) where they reviewed the AHS cohort data is significant. They found no association between glyphosate and NHL. The authors noted that the aforementioned Midwest USA case control studies were retrospective in design and therefore potentially susceptible to recall bias as regards exposure reporting.

The cross-Canada case – control study

The IWG reviewed a report by McDuffie et al, (2001) who studied the association between NHL and exposure to specific pesticides in a multicentre population-based study with 517 cases and 1,506 controls among men of six Canadian provinces. The authors reported a slight, non-statistically significant increased risk for NHL from claimed glyphosate exposure, the OR being 1.26 (95% CI 0.87–1.80) for analysis adjusted for age and province, and 1.20 (95% CI 0.83–1.74) for analysis adjusted for age, province and high-risk exposures. The study also assessed the significance of different exposure durations. When stratified by greater than or less than two days of glyphosate exposure/year (< 2d/year), the values were 2.12 (95% CI 1.20–3.73) for >2d/year relative to those with < 2d/year (assigned OR of 1.0). The authors commented that although there was not a statistically significant finding for exposure to glyphosate per se, there was a dose-response relationship.

Case-control studies in Sweden

The IWG reviewed a study by Eriksson et al, (2008) who reported the results of a population-based case-control study of exposure to pesticides as a risk factor for NHL. Men and women aged 18–74 years living in Sweden were included from 1 December 1999 to 30 April 2002. In total, 910 (91%) cases and 1,016 (92%) controls participated. The authors found NHL associations with exposure to glyphosate. This exposure was reported by 29 cases and 18 controls, giving a reported odds ratio of 2.02 (95% CI 1.10–3.71) in a multivariate analysis. When restricted to a >10 year latency period the OR became 2.26 (95% CI 1.16–4.40). Odds ratios were also reported for lymphoma subtypes. For only two of the eight subtypes were odds ratios statistically significant; likely related to the small numbers. The IWG considered that this was a large study; that there was possible confounding from the use of other pesticides including MCPA, but this was controlled for in the analysis. Given the number of cases studied for glyphosate (29 cases and 18 controls) this study could hardly be considered as large. Twelve subjects were in a less than 10 days exposure group and 17 in a more than 10 days group. Therefore this study had limited power to detect an effect.

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Other findings

In 2014 Schinasi and Leon reported their study of the association between NHL and occupational exposure to various agricultural pesticide chemical groups. Some findings on glyphosate were presented; for example the results from the studies by McDuffie et al, (2001), De Roos et al, (2005) and Eriksson et al, (2008) were given. This review included a series of meta-analyses, which they asserted showed consistent evidence of positive associations between NHL and carbamate insecticides, organophosphorus insecticides, lindane, and MCPA. As regards glyphosate (an "organophosphorus herbicide"), "in a handful of papers", associations between pesticides and NHL subtypes were reported; B cell lymphoma was positively associated with phenoxy herbicides and glyphosate.

The Agricultural Health Study (AHS) cohort studies

These studies in Ohio and North Carolina involve a large cohort of private and commercial pesticide applicators (57,311 as at 2004–5). Several studies have been conducted using this cohort.

Alavanja et al, (2003) evaluated associations between specific pesticides and prostate cancer in the AHS. Glyphosate was listed as one of the pesticides with sufficient exposure data for analysis, but the findings for it were not listed, so that it has been assumed that no significant positive association was found with prostate cancer.

Flower et al, (2004) evaluated associations between pesticide application by parents and cancer among children born to Iowa participants in the AHS. There was no positive association between either maternal or paternal use of glyphosate and risk of childhood cancer.

De Roos et al, (2005) evaluated associations between glyphosate exposure and "all cancers" or any cancer site using the AHS cohort. This study did not show a significantly increased risk of NHL. In the group reportedly exposed to glyphosate, small, non-statistically significant relative risks of 1.2 (95% CI 0.7–1.9) adjusted for age (only) and 1.1 (95% CI 0.7–1.9) adjusted for age, demographic and lifestyle factors and other pesticide exposure were found for NHL, (De Roos 2005). There was no dose (exposure) response relationship.

De Roos et al, (2005) also found a non-statistically significant association between glyphosate exposure and multiple myeloma, with rate ratios (RR values) of 1.1 (95% CI 0.5–2.4) adjusted for age only, and 2.6 (95% CI 0.7–9.4) adjusted for age, demographic and lifestyle factors and other pesticides exposures. Such a finding had not previously been reported.

Comparisons were made between ever-exposed versus never-exposed groups, and between three equal sized groups (tertiles), formed by subdivision either on the basis of total days of exposure or intensity-weighted exposure days. In the intensity-weighted analysis of glyphosate and lung cancer, the relative risk for the highest tertile was only 0.6 (95% CI 0.3–1.0), for pancreatic cancer the RR for the highest tertile was 0.5, while for multiple myeloma the RR was 2.1, but the confidence interval was wide (0.6–7.0). None of these findings reached statistical significance at 95%. Regarding the whole group (ie ever used glyphosate), the RR for multiple myeloma was 1.1 (95% CI 0.5–2.4) adjusted for age only, and 2.6 (95% CI 0.7–9.4) adjusted for age, demographic and lifestyle factors and other pesticide exposures. Unremarkable, non-statistically significant results were found for the other cancer sites assessed.

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Thus as regards this study, there was no evidence of a statistically significant positive association for any of the cancers for which data were reported (Mink et al, 2012). Furthermore De Roos et al, (2005) acknowledged in their paper that over 13,000 subjects were excluded from multivariate analyses because of missing data. In analyses of "ever" versus "never" exposed to glyphosate, the age-adjusted relative risk of multiple myeloma was 1.1. Lash (2007) assessed the study design and concluded that adjustment for confounders, which resulted in limiting the data set by 25% because of missing data on the adjustment variables, likely introduced selection bias, which was likely to have been in the direction away from the null (ie exaggerating any possible risk).

It is also known that multiple myeloma is often preceded by monoclonal gammopathy of undetermined significance (MGUS), a pre-malignant plasma cell disorder (Morgan et al, 2002). It is of interest to note that a decreased risk (albeit not statistically significant) of MGUS was observed in glyphosate applicators in the AHS.

Engel et al, (2005) evaluated breast cancer risk among wives of farmers in the AHS. No statistically significant association was found.

In an analysis of colorectal cancer and pesticide use, Lee et al, (2007) found no statistically significant association between glyphosate use and cancer of the colon or rectum.

Andreotti et al, (2009) reported no significant association of "ever" use (versus "never use") of glyphosate with pancreatic cancer among the combined group of AHS applicators and spouses (OR 1.1; 95% CI 0.6–1.07), nor was there evidence for a dose-response relationship.

Dennis et al, (2010) evaluated associations of 50 pesticides with cutaneous melanoma in the AHS cohort. Glyphosate was listed as one of the 22 pesticides on the enrolment questionnaire. The authors commented that none of these 22 pesticides was associated with melanoma.

None of the AHS cohort study analyses reported statistically significant positive findings for glyphosate exposure and total cancer or any site-specific cancer, in adults or children. In particular, the prospective AHS studies did not corroborate the positive association with NHL reported by the Swedish case-control studies. Analyses of increasing category of glyphosate exposure days and incidence of NHL produced rate ratios that were below the null value of 1.0 (De Roos et al, 2005 and Mink et al, 2012).

Discussion of review of epidemiological findings

In a review of glyphosate in 2006, the WHO observed that:
"widely used pesticides, like glyphosate, have recently become a focus of epidemiological research. In the past few years several epidemiological studies have been published that reported weak associations of glyphosate with lymphopoietic cancers, self-reported adverse reproductive outcomes and self-reported attention deficit hyperactivity disorder in children. However, the results of these studies do not meet generally accepted criteria from the epidemiology literature for determining causal relationships. Generally, the associations were rather weak and rarely statistically significant. Controlling for potential confounding factors, including other pesticides exposure, was not possible owing to limited available information and small numbers of subjects".

Whether or not there was any internal exposure or the extent of such exposure was not measured and, accordingly, a possible dose–response relationship could not be evaluated.

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This seems a fair assessment of several of the studies regarding glyphosate and its formulations. De Roos et al, (2005) noted that the Midwest USA case control studies were retrospective in design and therefore potentially susceptible to recall bias as regards exposure reporting. Certainly a large prospective cohort study (such as that by De Roos et al, 2005) is much preferable to smaller case-control studies, the latter of which have much less statistical power to identify causal associations and are subject to more biases, including those regarding exposure assessment. Therefore much more weight should be given to the De Roos et al, (2005) cohort study than the much smaller De Roos et al, (2003) case-control study. In that regard, it is important to note that the cohort study found no association between glyphosate and NHL. There was, however, a small (non-statistically significant) increased risk of multiple myeloma in the 2005 study, but the point estimates of this risk may have been exaggerated. (Lash 2007.)

A re-analysis of some data from the De Roos et al, (2005) study has recently been undertaken, with a focus on multiple myeloma (Sorahan, 2015). Assessing the same data, Sorahan found no significant trends of multiple myeloma risk with reported cumulative days of glyphosate use, and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analysis had made adjustment for a few basic variables (age and gender) or made adjustment for many other lifestyle factors or pesticide exposures; as long as data on all available pesticide applicators was used.

Sorahan (2015) argued that the elevated rate ratios (or relative risks) for multiple myeloma reported previously by Roos et al, (2005) arose from use of restricted data sets that, probably by chance, turned out to be unrepresentative. These restrictions were considered to be unnecessary and undesirable, as potentially informative data on the exposure or outcome under investigation were discarded. For example, it was asserted that there were a number of lost cases of multiple myeloma in the group of applicators who had never used glyphosate, because they were excluded by Roos et al, (2005) due to their not having data on for example use of alcohol, or smoking. These lost cases in the baseline category gave a false impression of elevated rates in ever-users. As a result Sorahan gave more weight to the point estimate of 1.1 as the RR (adjusted for age only) as opposed to the estimate of 2.6 as the RR for ever-use of glyphosate (adjusted for age, demographic and lifestyle factors, and other pesticides).

Mink et al, (2012) reviewed the epidemiological literature (and relevant methodological and biomonitoring studies) to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. Seven cohort studies and fourteen case-control studies examining a potential association between glyphosate and one or more cancer outcomes were subjected to a qualitative analysis.

The cohort studies were all based on analyses of participants or family members of the AHS cohort. Mink et al (2012), observed that none of the AHS cohort study analyses reported statistically significant positive findings for glyphosate exposure and total cancer or any site-specific cancer in adults or children. They found no consistent pattern of positive associations to suggest a causal relationship between human exposure to glyphosate and any cancer.

Overall, this 2012 review found no consistent pattern of positive associations between total cancer (in adults or children) or any site-specific cancer, and exposure to glyphosate. They suggested a cautious interpretation of the few positive associations reported, and concluded that the epidemiological data, when considered together, did not support a causal association between glyphosate exposure and cancer.

Similarly, the latest report of BfR (2015) to the European Food Safety Authority (EFSA)[1] based on the evaluation of over 30 epidemiological studies came to the overall assessment that there is no validated or significant relationship between exposure to glyphosate and an increased risk of NHL or other types of cancer.

A recent peer review by EFSA[2] (2015) essentially confirmed the conclusions in their re-evaluation of glyphosate. They noted that 10 cohort studies (which included the AHS, the largest series of prospective studies to date), found that glyphosate did not cause different types of cancer and did not increase risk of all cancers combined. (As noted earlier, the findings for NHL were negative in the AHS cohort.) Similarly nine case-control studies did not indicate an increased risk of carcinogenicity, or did not have sufficient power to assess this. With regard to NHL, the case-control studies exhibited poor consistency in their results and small numbers of cases limiting the statistical significance of findings in some studies. As noted above, case-control studies have less power, are more subject to various biases, and are less effective at assessing actual exposure levels than are cohort studies. EFSA concluded that there is very limited evidence for an association between glyphosate exposure and the occurrence of NHL.

Cancer in experimental animals

Mice studies

Glyphosate was tested in female and male mice by dietary administration in two studies. A skin application in one initiation-promotion study was conducted with male mice.

The IWG found that in male CD-1 mice, glyphosate induced a positive trend in the incidence of a rare tumour, renal tubule carcinoma. A second study reported a positive trend for hemangiosarcoma in male mice. A glyphosate formulation promoted skin tumours in an initiation-promotion study in mice.

The IWG noted there was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in male CD-1 mice in a glyphosate feeding study (0, 1,000, 5,000, or 30,000 ppm glyphosate *ad libitum* for 24 months). (This study was conducted prior to the institution of GLP.) The study was submitted to the US EPA which requested that a pathology working group (PWG) be convened to evaluate the renal tumours. In this second evaluation, the PWG found that the incidence of adenoma was not statistically significant but the incidence of carcinoma and the incidence of adenoma and carcinoma (combined) were significant. The IWG considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate.

However, this finding is at variance with the US EPA (1993) which reported in their glyphosate review that the occurrence of these adenomas was spontaneous rather than compound-induced because the incidence of renal tubular adenomas in males was not statistically significantly different when compared with the concurrent controls. An independent group of pathologists and biometricians also conducted extensive evaluations of these adenomas and reached the same conclusion. The US EPA concluded glyphosate was not considered to be carcinogenic in this study.

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[1] The BfR (2015) report addressing the carcinogenicity of glyphosate is a report of Germany specifically, as Germany was the lead member state for the EFSA review of glyphosate.

[2] EFSA accepted the conclusion relating to glyphosate and cancer (including NHL), with one dissenting member state.

The IWG reviewed a second feeding study reported to the FAO/WHO Joint Meeting on Pesticide Residues (JMPR), and found there was a significant positive trend in the incidence of hemangiosarcoma in male CD-1 mice. Groups of 50 female and male mice were fed diets containing glyphosate at a concentration that was adjusted weekly for the first 13 weeks and every four weeks thereafter to give doses of 0, 100, 300, or 1,000 mg/kg body weight, *ad libitum* for 104 weeks.

In contrast JMPR (WHO 2006) found that owing to the lack of a dose-response relationship, the lack of statistical significance and the fact that the incidences recorded in this study fell within the historical ranges for controls, these changes were not considered to be caused by administration of glyphosate. They concluded administration of glyphosate to CD-1 mice for 104 weeks produced no signs of carcinogenic potential at any dose.

Initiation-promotion

The IWG found that in a study involving 20 male Swiss mice which had a glyphosate based formulation applied to their skin, it appeared to be a tumour promoter, but they concluded that this was an inadequate study because its design was poor, with short duration of treatment, no solvent controls, small numbers of animals, and a lack of histopathological examination.

However the BfR (2015) considered that generally testing of formulations should not be used for the toxicological evaluation of active substances because co-formulants may extensively alter the outcome. The BfR deemed that this IWG finding was not considered by the institutions in the EU to be evidence for the carcinogenic properties of glyphosate per se.

Review articles – mice studies

The IWG noted that Griem et al, (2015) had published a review article which included discussion of five long-term glyphosate feeding studies in mice. Two of the studies were discussed in the IARC monograph. The working group summarised the other three studies but claimed that it was unable to fully evaluate the other three studies because of the limited experimental data provided in the review article and supplemental information.

Griem et al, (2015) noted that the five mouse studies that they reviewed were submitted to support glyphosate renewal in the EU. They considered that all but the oldest study were reliable without restriction and were performed under conditions of GLP and OECD protocols.

During the EFSA peer-review process for the renewal of the approval of glyphosate, EFSA also received a complementary mandate from the EU to consider the findings by IARC regarding the potential carcinogenicity of glyphosate (EFSA 2015).

The EFSA peer review (2015) also evaluated the five mice studies. Only one of these suggested a potential carcinogenic effect, as evidenced by a statistically significant increased evidence of malignant lymphomas at the top dose level of 1,460 mg/kg/day. However the validity of the study was questioned, due to the occurrence of viral infection which could have influenced survival rates and the incidence of lymphomas. No carcinogenic effects were observed at the highest dose levels in any of the other studies. The IWG evaluated two of these studies and asserted positive trends in males for renal tubular carcinomas in one study and for hemangiosarcoma in the other. However EFSA took a weight-of-evidence approach; with considerations including the statistical significance being only found in trend analysis but not in pairwise comparison, lack of consistency in multiple

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animal studies, the fact that the slightly increased incidences only occurred at doses higher than those recommended for the oral route in carcinogenicity studies, incidences in test animals generally being within the historical range for control groups, and the lack of pre-neoplastic lesions.

Rat studies

Five feeding studies in rats and two drinking water studies with glyphosate were reviewed by the IWG.

Drinking water

One study in Sprague-Dawley rats was considered by the IWG to be inadequate for evaluation because of its short exposure duration.

A glyphosate containing drinking water study with Wistar rats did not show any significant increase in tumour incidence.

Dietary administration

Two studies in Sprague-Dawley rats showed a significant increase in the incidence of pancreatic islet cell adenoma in male rats. One of these studies also showed a significant positive trend in the incidence of hepatocellular adenoma in males and of the thyroid C-cell adenoma in females. However two studies (one in Sprague-Dawley and one in Wistar rats) found no significant increase in tumour incidence at any site.

The IWG reviewed a chronic feeding study (provided by the US EPA) in which groups of 60 female and male Sprague Dawley rats were given diets containing glyphosate at a concentration of 0, 2,000, 8,000 or 20,000 ppm *ad libitum* for 24 months. In males at the lowest dose, there was a statistically significant increase in the incidence of pancreatic islet cell adenoma compared with controls. Additional analyses by the US EPA revealed a statistically significant higher incidence of pancreatic islet cell carcinoma in males at the lowest and highest doses compared with controls: lowest dose, 8/45 (18%); intermediate dose, 5/49 (10%); highest dose, 7/48 (15%) versus controls, 1/43 (2%). The range for historical controls for pancreatic cancer islet cell carcinoma reported in males at this laboratory was 1.8–8.5%. The IWG concluded that this study demonstrated a significant increase in the incidence of pancreatic islet cell adenoma in male rats.

However the US EPA (1993) had concluded that:
"these adenomas were not treatment-related and glyphosate was not considered to be carcinogenic in this study. With respect to pancreatic islet cells adenomas, there was no statistically significant positive dose-related trend in their occurrence; there was no progression to carcinomas; and the incidence of pancreatic hyperplasia (non-neoplastic lesion) was not dose-related. With respect to hepatocellular adenomas, the increased incidence of these neoplasms was not statistically significant in comparison with the controls; the incidence was within the historical control range; there was no progression to carcinomas; and the incidence of hyperplasia was not compound-related. With respect to thyroid C-cell adenomas, there was no statistically significant dose-related trend in their occurrence; the increased incidence was not statistically significant; there was no progression to carcinomas; and there was no significant dose-related increase in severity or incidence of hyperplasia in either sex".

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Also, in the JMPR (WHO 2006) review of this study they reported:
"The historical-control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of studies reported recently in the literature revealed a prevalence of 0–17% in control males with several values being ☐8%. More importantly, the incidences of islet cell adenomas clearly did not follow a dose-related trend in the treated groups of males. There was no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a male in the control group, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support the conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to administration of glyphosate".

Review articles – rat studies

The IWG noted that Griem et al, (2015) had published a review article containing assessments of nine long-term glyphosate feeding studies in rats. Five of these studies were reviewed by the IWG. The remaining four studies were not evaluated by the IWG which stated that there was limited experimental data provided in the review article. These four studies had been submitted to various organisations for registration purposes. There was no evidence of a carcinogenic effect related to glyphosate treatment.

Its long-term toxicity and carcinogenicity was assessed in nine rat studies. The EFSA peer review concluded that no significant increase in tumour incidence was apparent. Three of these studies were not evaluated by the IARC panel. In two studies, increased incidences of pancreatic islet cell adenomas were found but were not dose-related. EFSA also noted that the significance of these findings depended on the statistical analysis: using a pairwise comparison (as planned for in the study protocol) no significant effect is observed, whereas a trend analysis performed by the IWG identified significant changes. EFSA noted that deviations from the statistical analysis used by the study authors should be limited and properly justified.

Other relevant data

The IWG group noted that soil microbes degrade glyphosate to aminomethylphosphonic acid (AMPA). Blood AMPA detection after glyphosate poisoning incidents suggests intestinal microbial metabolism in humans.

Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Neimann et al, (2015) published a critical review and comparison of data obtained in a total of seven studies from Europe and the US. They concluded that no health concern was revealed because the resulting exposure estimates were several magnitudes lower than the acceptable daily intake (ADI) or the acceptable operator exposure level (AOEL).

The measured internal exposure was clearly below the worst-case predictions made in the evaluation of glyphosate as performed for the renewal of its approval within the European Union.

This is consistent with the risk-based approach that regulatory agencies use when considering realistic dosages and real-life conditions. Those studies show that farmers and farm families are exposed to significantly lower doses of the herbicide than some model estimates would suggest.

It is also in keeping with an earlier review (Williams et al, 2000) of the animal data, in which dose levels from animal toxicity tests were compared to conservative, upper-limit estimates

of human exposure to glyphosate, to give a margin of exposure (MOE) value. MOE analyses compare the lowest NOAELs determined from animal studies to worst-case levels of human exposure; with MOEs of greater than 100 indicating confidence that no adverse health effects would occur. These authors found in their review that the MOEs for worst-case chronic exposure to glyphosate ranged from 3,370 to 5,420, and concluded that "under present and expected conditions of use, Roundup herbicide does not pose a health risk to humans".

Genotoxicity

The IWG claimed that there is strong evidence that glyphosate is genotoxic. They tabulated numerous reports of tests relating to the genotoxicity of glyphosate and its formulations, with some showing a positive association, and some a negative association.

The evaluation of the large volume of genotoxicity data available requires consideration of assay system validation, test system species used, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose-response, and relationship of effects to toxicity. The guidelines for genetic toxicology tests developed for the OECD are a pre-eminent source of internationally agreed guidelines.

There were often inconsistent results reported (both positive and negative) from the same test systems in different laboratories. The relevance of many of the assays in test system species (fish, oysters, insects, snails, worms and caimans) which have never been validated for the assessment of genotoxicity in humans for regulatory purposes, is questionable. Additionally the *intraperitoneal* route of exposure for many of the mammalian *in vivo* studies is not appropriate since it does not reflect normal human exposure, with doses exceeding occupational exposure by orders of magnitude.

Kier and Kirkland (2013) published a review of the genotoxicity of glyphosate and glyphosate-based formulations. This review concluded that there was a strong weight of evidence that glyphosate and its formulations are predominantly negative in well-conducted, core bacterial reversion and *in vivo* mammalian micronucleus and chromosomal aberration assays. Although some positive results for glyphosate and glyphosate-based formulations were reported in DNA damage assays, and for the micronucleus endpoint for formulations in non-mammalian studies, the positive results were associated with high dose levels and/or overt toxic effects. The preponderance of negative results in core assays supports the conclusion that reports of DNA damage or non-mammalian micronucleus effects are likely to be secondary to cytotoxicity rather than indicative of DNA-reactive mechanisms.

The IWG found that glyphosate and glyphosate formulations induced DNA and chromosomal damage in mammals, and in human and animal cells *in vitro*. They referred to one study (Bolognesi, 2009) reporting increases in blood markers of chromosomal damage (micronuclei) in residents of several communities after spraying of glyphosate formulations, to support this contention of genotoxicity.

However, the authors of the Bolognesi (2009) study concluded that overall, data suggesting that genotoxic damage (as evidenced by the micronuclei test) associated with glyphosate spraying for control of illicit crops is slim, and any such effect appears to be transient. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for coca and poppy eradication is low. The attribution of a genotoxic effect due to glyphosate exposure rather than a multitude of other demographic and environmental causes seems rather tenuous given the uncertainty of actual exposure.

In a recent communication, EFSA summarised their appraisal of the genotoxicity studies. *In vitro* tests of mutagenicity gave consistently negative results. *In vitro* tests of mammalian chromosome aberration (all of those which had been performed under GLP conditions) were also negative. Positive results were found in some published *in vitro* studies of chromosomal aberrations, but these were not confirmed by *in vivo* studies addressing the appropriate endpoints, such as the micronucleus test.

As regards *in vivo* tests, all studies conducted according to internationally validated guidelines for good laboratory practice (GLP) and some non-GLP published studies gave negative results. Two non-GLP studies were positive in mice treated intraperitoneally, but at levels close to or above the LD_{50}[3] (possibly suggestive that this is a secondary effect), and one study had major flaws. No genotoxic effects on germ cells have been detected in rats or mice treated orally at dose levels up to 2,000 mg/kg/day (the maximum dose level recommended for such studies). EFSA concluded that, considering the weight of evidence, glyphosate is unlikely to be genotoxic *in vivo*.

As regards glyphosate-based commercial formulations, a number of formulations with unknown composition have given positive results when tested *in vitro* and *in vivo*. However some of the test systems are not validated and/or interpretation is difficult due to possible confounding, such as cytotoxicity, specific organ toxicity or unclear relevance to humans (such as tests in fish, amphibians, or invertebrates). Some of the co-formulants (such as polyethoxylated tallow amine (often abbreviated to POEA)) may be more systemically toxic than glyphosate. However EFSA concluded that the genotoxic potential of such complete formulations should be further assessed.

Kier (2015) reviewed genotoxicity biomonitoring studies of glyphosate-based formulations. He found that most of the human biomonitoring studies were not informative because there was either a very low frequency of exposure to glyphosate formulations or exposure to a large number of pesticides in addition to glyphosate without analysis of specific pesticide effects. One pesticide sprayer biomonitoring study indicated there was no statistically significant relationship between frequency of exposure to glyphosate formulations reported for the last spraying season and oxidative DNA damage. There were three studies of human populations in regions of glyphosate formulation aerial spraying. One study found increases for the cytokinesis-block micronucleus endpoint but these increases did not show statistically significant associations with self-reported spray exposure and were not consistent with application rates. A second study found increases for the blood cell comet endpoint at high exposures causing toxicity. However, a follow-up to this study two years after spraying did not indicate chromosomal effects.

Oxidative stress

The IWG found that glyphosate, glyphosate formulations, and AMPA induced oxidative stress in rodents and *in vitro*.

Oxidative stress was only found in one study in rats administered intraperitoneal glyphosate active ingredient (Astiz et al, 2009), and in numerous studies using *intraperitoneal* administration or *in vitro* methods with glyphosate-based formulations. However, these studies used doses that exceeded normal occupational exposures by orders of magnitude and the *intraperitoneal* route of exposure is not appropriate for evaluating human exposure. Glyphosate has low gastrointestinal absorption and poor dermal absorption. It therefore

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[3] LD_{50} is the dose of the substance required (usually expressed in relation to body weight) that is estimated to kill 50% of the test population.

seems unlikely that human exposure would produce the sort of tissue levels used in the oxidative stress tests. There was also some inconsistency in results.

Most effects were seen when whole glyphosate formulations were tested. EFSA considered that generally testing of formulations should not be used for the toxicological evaluation of active substances because co-formulants may extensively alter the outcome. Thus any effects found cannot then be attributed to the glyphosate active ingredient present.

Discussion

The IARC WG (IWG) classified glyphosate as "probably carcinogenic to humans (Group 2A)" as the overall evaluation.
As set out in their evaluation section, this was based on:

  ☐ "*limited evidence*" in humans for the carcinogenicity of glyphosate, and

  ☐ "*sufficient evidence*" in experimental animals for carcinogenicity of glyphosate.

The rationale identifies that the IWG also notes mechanistic and other relevant data in support of the conclusion; in particular the IWG cites "strong evidence" that glyphosate can operate by two key characteristics of known human carcinogens, namely genotoxicity and oxidative stress.

This discussion section of the report will consider each of these sources of evidence in turn as contributing factors to the IWG's overall evaluation.

Human epidemiological evidence

The key cited studies in support of the "limited evidence" in humans for carcinogenicity of glyphosate consisted of three case-control investigations. The odds ratios (OR) for cases of NHL and glyphosate exposures are summarised in the following table.

Odds ratios (OR) for cases of NHL and glyphosate exposures

Study area	OR[1] and 95% CI[2]	Study reference
Midwest, USA	2.1 (1.1–4.0) [logistic regression] 1.6 (0.9–2.8) [hierarchical regression]	De Roos et al, 2003
Canada	1.26 (0.87–1.8) 1.20 (0.83–1.74) [adjusted for medical variables]	McDuffie et al, 2001
Sweden	2.02 (1.1–3.71) [univariate] 1.51 (0.77–2.94) [multivariate]	Erikson et al, 2008

1. OR is the odds ratio of outcome of interest between the relevant case group and the reference or control group.
2. The 95% CI are the confidence intervals round the OR representing the limits within which there is 95% confidence that the true value falls.

The first important observation is that depending on the statistical tests used only two studies (Midwest USA and Sweden) show OR values indicating statistical significance at the 95% level. In the Midwest USA, however, this is only true using logistic regression, while in the Swedish study only the univariate analysis showed statistical significance.

Some case control studies assessed data using dose (exposure)/response or intensity/response to determine whether or not there is a trend to a higher incidence of tumours in persons categorised as having higher exposures to glyphosate. While these approaches are desirable, the criteria of exposure seem low. For one case-control study, the criterion for high or lower glyphosate use was greater than or less than two days of glyphosate use/year (McDuffie et al, 2001), whereas in another the criterion was greater than or less than 10 days of glyphosate use/year (Eriksson et al, 2008). While the distribution of use category was not given in either study, 2–10 days of use per year seems a low benchmark for exposure comparisons. The direct glyphosate exposure findings with respect to NHL was not significant in the McDuffie et al, 2001 study, but they reported a dose response based on this dose comparison and quoted the OR for exposure >2 day/year as 2.12 (95% CI 1.20–3.73).

The direct glyphosate exposure findings with respect to NHL were significant in the Swedish study using univariate evaluation, and the effect of dose-response in the Swedish study appears to only be statistically significant using this approach (considering the data presented in the IARC Monograph in Table 2.2, p23) which reported a higher OR for "heavy" users (>10 days/year) of 2.36 (95% CI 1.04–5.37). It is noteworthy that the paper reports the highest OR, 2.81 (95% CI 1.27–6.22), for the association between exposure to MCPA and NHL. This may be the explanation for the difference between the results using univariate and multivariate evaluation. When considering the latency period, >10 years exposure to glyphosate had an OR of 2.26 (95% CI 1.16–4.4) in comparison to ≤ 10 years with an OR of 1.11 (95% CI 0.24–5.08), but these findings may be confounded by exposure to MCPA or other phenoxy herbicide exposures. There could be residual confounding from MCPA exposure if the participants under-reported earlier MCPA exposure. The apparent increased risk with latency for glyphosate exposure could be because participants who had sprayed pesticides for longer were more likely to have used the phenoxy herbicides (including MCPA) earlier in their working lives.

The AHS cohort study (De Roos, et al, 2005) had a more detailed assessment at different exposure intensities as they used cumulative lifetime days of use and an intensity measure (years of use x days/year x estimated exposure level). The data (presented in Table 2.1 of the IARC Monograph on p12) for this cohort study showed no statistically significant difference for the trend to increased exposure with exposure bands at 0–20, 21–56 and 57–2,678 cumulative days of exposure, despite the higher exposure levels in comparison to the case-control studies.

It is important in these circumstances to consider the overall data set. Rather than only highlighting the three case-control studies which identified a marginally statistically significant association between reported glyphosate use and NHL, the overall assessment needs to take into account other studies which did not demonstrate such an association. Also, it is particularly important to note the lack of significant finding in a large cohort study (the AHS) where the potential for recall bias is greatly reduced and should therefore be given greater weight than the case control studies. Cohort studies are generally considered more reliable than case-control studies, because the population is defined and the exposure parameters and the potential confounding exposures and lifestyle factors are established prior to the adverse outcome of interest so that the potential for recall bias is less likely.

Given the lack of confirmation of the small number of positive findings from case-control studies in the more powerful cohort study, the epidemiological support for the conclusion "limited evidence" in humans is not convincing.

Experimental animal studies

The key cited studies in support of the "sufficient evidence" in experimental animals for carcinogenicity of glyphosate consisted of three studies in mice. These comprised one oral study demonstrating a positive trend for increased incidence of renal tubule carcinoma, one oral study in mice demonstrating a positive trend for increased incidence of hemangiosarcoma; and a supporting skin study demonstrating tumour promotion using a glyphosate formulation. In addition, one rat study demonstrated an increased incidence of pancreatic islet cell adenomas.

In assessing these data, the IWG used different statistical tests to those in the original analysis (trend analysis rather than a pairwise comparison against controls). The original studies were designed with the intention to assess statistical significance by means of a pairwise comparison between the test and control groups, so use of the trend assessment by IARC to assess these data requires justification. IARC's use of the trend assessment gave a positive response, but in none of the studies are the positive effects statistically significant using the original statistical approaches. Also, the IWG did not take into account the generally accepted assessment of the same data by international panels of experts, which took into account additional historical incidence data for hepatocellular adenomas in the rats and the presence of a viral infection in the mouse study which could have influence survival rates and the incidence of lymphomas.

The promotion study using a glyphosate-based formulation should not be used as support for the carcinogenicity of glyphosate per se, since the test substance contains other components which might influence the outcome.

The IWG did not evaluate some other studies which have been used by other regulators. These did not support the view that exposure to glyphosate in long-term feeding studies was associated with an increase in tumours at any sites. While the IWG approach is consistent with the IARC pre-amble and policy on the selection of study data, in the current circumstances this attributes inappropriate weight to the three studies which IWG considered and for which their analysis found an increase in tumours. Firstly because other studies which other reputable bodies found to be negative were not considered, and secondly because the reasons why the above findings were not relied upon by other assessments were not taken into account by the IWG. In particular a lack of consistency (dose-response) in multiple studies, slight increases in incidence at the maximum tested dose only, or incidences within the historical control range.

Taking into account that the positive findings cited by the IWG were not assessed as evidence of a carcinogenic effect in the view of other reputable bodies, and that the total data set of long-term carcinogenicity bioassays were consistently negative, it is concluded that the overall weight of evidence does not indicate that glyphosate is carcinogenic.

Mechanism of action

The IWG cites what is described as "strong evidence" that glyphosate can operate by two key characteristics of known human carcinogens – genotoxicity and oxidative stress.
The studies used in support of this conclusion were primarily *in vitro* mammalian cell studies. In such studies the mammalian cells are directly exposed to the test substance (glyphosate or a glyphosate-based formulation) at high concentrations which would not be reasonably achieved in an *in vivo* exposure whether in animals or humans. All studies done according to internationally validated guidelines gave negative results, while studies using unvalidated

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test method/species, or with glyphosate-containing formulations or using high *intraperitoneal* doses are inappropriate for assessment of genotoxicity to humans.

Other supporting evidence for this conclusion included DNA damage and micronuclei in various populations allegedly exposed to glyphosate from sprays. Attributing the effects found to the exposure to glyphosate is questionable when the exposure, if any, was to glyphosate-based formulations and unidentified demographic, geographical or lifestyle factors that could be responsible for the DNA damage.

In relation to oxidative stress this was only found in one study in rats administered *intraperitoneal* glyphosate active ingredient (Astiz et al, 2009), and in numerous studies using *intraperitoneal* administration or *in vitro* methods with glyphosate-based formulations. The *intraperitoneal* route of administration is not considered relevant to human exposures. Glyphosate has low gastrointestinal absorption and poor dermal absorption. There was also some inconsistency in results. So the evidence for glyphosate causing oxidative stress is considered weak.

Conclusion

The overall conclusion is that – based on a weight of evidence approach, taking into account the quality and reliability of the available data – glyphosate is unlikely to be genotoxic or carcinogenic to humans and does not require classification under HSNO as a carcinogen or mutagen.

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